

CLAIMS

1/ Polypeptides, characterized in that they include at least 3 proteins or any part of one or more of these proteins necessary to confer on Gram-positive bacteria resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin, or of promoting this resistance, in particular in strains of the family of the Gram-positive cocci, these proteins or parts of proteins being recognized by antibodies directed against one of the sequences identified in the list of sequences by

SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3.

2/ Polypeptides according to Claim 1, characterized in that they correspond to the combination of the proteins designated by SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3.

3/ Polypeptides according to one or other of the Claims 1 or 2, characterized in that the amino acid sequences necessary for the expression of resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin, are under the control of regulatory elements, in particular proteins corresponding to the sequences designated by SEQ ID NO 4 or SEQ ID NO 5 in the list of sequences.

4/ Polypeptides according to any one of the Claims 1 to 3, characterized in that they are encoded in the sequences SEQ ID NO 6 identified in the list of sequences.

5/ Purified protein characterized in that it corresponds to the sequence SEQ ID NO 2 contained in the polypeptides according to Claim 1 or Claim 2.

6/ Protein characterized in that it corresponds to one of the sequences identified by SEQ ID NO 1, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5.

7/ Nucleotide sequence characterized in that it codes for an amino acid sequence according to any one of the Claims 1 to 6.

8/ Nucleotide sequence of about 7.3 kb corresponding to the HindIII-EcoRI restriction fragment such as that obtained from the plasmid pIP816, consisting of this HindIII-EcoRI fragment or any part of this fragment, in particular the EcoRI-XbaI fragment of 3.4 kb, the EcoRV-SacII fragment of about 1.7 kb and the HindIII-EcoRI fragment of 3.3 kb.

9/ Nucleotide sequence according to Claim 8, characterized in that it contains the following restriction sites such as those obtained from the plasmid pIP816 in the following order :

HindIII, BglII, BglIII, EcoRI, BamHI, XbaI, EcoRI.

10/ Nucleotide sequence according to either of the Claims 8 or 9, characterized in that it corresponds to the sequence identified by SEQ ID NO 7, or in that it includes this sequence or any part of this sequence susceptible :

- either of constituting a hybridization probe for the detection of resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin, especially in strains of the family of the Gram-positive cocci,
- or of coding for a sequence necessary for the expression of resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin, especially in strains of the family of the Gram-positive cocci.

11/ Nucleotide sequence according to any one of the Claims 8 to 10, characterized in that it is one of the sequences SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10., or a variant of one of these sequences provided that it codes for a protein having immunological and/or functional properties similar to those of the proteins encoded in the sequences SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10..

12/ Nucleotide sequence according to any one of the Claims 7 to 10, characterized in that it corresponds to the sequence SEQ ID NO 6 or in that it includes this sequence.

13/ Recombinant sequence, characterized in that it comprises a nucleotide sequence according to any one of the Claims 7 to 12.

14/ Recombinant vector, characterized in that it comprises a nucleotide sequence according to any one of the Claims 7 to 13, at a site inessential for its replication under the control of regulatory elements capable of being implicated in the expression of resistance to antibiotics of the glycopeptide family, in particular vancomycin or teicoplanin, in a specific host.

15/ Recombinant vector according to Claim 14, characterized in that it is the plasmid pAT214.

16/ Recombinant cell host, characterized in that it comprises a nucleotide sequence according to any one of the Claims 7 to 13 or a vector according to Claim 14 or Claim 15 under conditions permitting the expression of resistance to antibiotics of the glycopeptide family, in particular vancomycin or teicoplanin, this host being for example selected from among the bacteria, in particular the Gram-positive cocci.

17/ Nucleotide probe, characterized in that it is capable of hybridizing with a sequence according to any one of the Claims 7 to 12, this probe being labelled if necessary.

18/ Nucleotide probe according to Claim 17, characterized in that it is specific in Gram-positive bacteria for sequences coding for a protein responsible for resistance to glycopeptides, in particular to vancomycin and/or teicoplanin and recognizes all of these sequences.

19/ Nucleotide probe according to Claim 17, characterized in that it is specific for a nucleotide sequence coding for a protein necessary for the expression of high-level resistance to antibiotics of the glycopeptide family, in particular to vancomycin and teicoplanin in Gram-positive bacteria.

20/ Nucleotide probe according to Claim 17, characterized in that it is specific for a nucleotide sequence coding for a protein necessary for the expression of low-level resistance to antibiotics of the glycopeptide family, in particular to vancomycin and teicoplanin in Gram-positive bacteria.

21/ Nucleotide probe according to any one of the Claims 17 to 20, characterized in that it hybridizes with a non-chromosomal nucleotide sequence of a strain resistant to glycopeptides, in particular vancomycin and/or teicoplanin, in particular in that it hybridizes with a non-chromosomal nucleotide sequence of a strain of Gram-positive cocci, for example a strain of enterococci and, preferably, E. faecium 4147.

22/ Monoclonal or polyclonal antibodies, characterized in that they recognize the polypeptide complex according to any one of the Claims 1 to 10 or an amino acid sequence according to any one of the Claims 1 to 6.

23/ Kit for the in vitro diagnosis of the presence of strains resistant to the glycopeptides, in particular to vancomycin and/or teicoplanin, these strains belonging in particular to the Gram-positive cocci, in particular in that they are enterococcal strains, for example E. faecium, characterized in that it contains :

- antibodies according to claim 22, labelled if necessary,
- a reagent for the detection of an immunological reaction of the antigen-antibody type.

24/ Kit for the in vitro diagnosis of the presence of strains resistant to the glycopeptides, resistant in particular to vancomycin and/or teicoplanin, these strains belonging in particular to the Gram-positive cocci, in particular they are strains of enterococci, for example E. faecium, characterized in that it contains :

- a nucleotide probe according to any one of the Claims 17 to 21, and if necessary,
- oligonucleoside triphosphates dATP, dCTP, dTTP, dGTP,
- an agent for the polymerization of DNA.

25/ In vitro detection procedure for the presence of strains resistant to the glycopeptides, in particular to vancomycin and/or teicoplanin, these strains belonging in particular to the family of the Gram-positive cocci, in particular in that they are strains of enterococci, for example E. faecium, characterized in that it comprises :

- a) the placing of a biological sample likely to contain the resistant strains in contact with a primer constituted by a probe according to any one of the Claims 17 to 21, capable of hybridizing with a desired nucleotide sequence necessary for the expression of resistance, this sequence being used as matrix in the presence of the 4 different nucleoside triphosphates and a polymerization agent under conditions of hybridization such that for each nucleotide sequence that has hybridized with a prime, an elongation product of the primer complementary to the matrix is synthesized,
- b) the separation of the matrix and the elongation product obtained, this latter then also being capable of behaving as a matrix,
- c) the repetition of step a) so as to produce a detectable quantity of the desired nucleotide sequences,
- d) the detection of the product of amplification of the nucleotide sequences.